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ROBERT F. PITTS MEMORIAL LECTURE

Mechanisms coupling the absorption of solutes and water in the proximal nephron[1]

In 1978, the many friends and admirers of Professor Robert Pitts established a fund to honor the memory of that distinguished physiologist. Subsequently, upon recommendation of its Renal Commission, with Klaus Thurau as chairman, the Council of the International Union of Physiological Sciences founded the Robert F. Pitts Lecture series, which is to be held every 3 years at the time and place of the Union's international congress. The lecturer is to be chosen by members of the Renal Commission and the Chairman of Physiology at Cornell University Medical College (ex officio). The first Lectureship was awarded to Professor Rolf Kinne, who delivered the lecture at the XXVIIIth Congress in Budapest in 1980.

Dr. Pitts was the "total" physiologist: investigator, teacher, and writer. He was justly famous for his precise experiments, based on impeccable logic, executed with precision, and reported succinctly. He was also a renowned teacher and the author of a renal physiology textbook, which set the standard in that field. Medical students at Cornell vividly recall Dr. Pitts' extensive, personal role in teaching the Medical Physiology course. Despite his enormous interest and productivity in research, he always seemed to choose teaching as his first professional obligation. He spent endless hours in lectures and conferences, appearing to relish the opportunity for animated discussions with small groups of students during teaching laboratories. For Robert Pitts, science had no national boundaries; his laboratory and department were filled with representatives from all corners of the globe, and his tutelage spawned professorships in numerous countries. It is singularly fitting, therefore, that the lectures in his memory should be held at international meetings.

***Dr. James A. Schafer,** the second Robert F. Pitts Lecturer, presented the following lecture in Sydney, Australia, August 29, 1983, Dr. Schafer completed his doctoral work under Dr. John A. Jacquez at the University of Michigan. He extended that work with a postdoctoral fellowship with Dr. Erich Heinz, and then with a long and fruitful association with Dr. Thomas Andreoli, first at Duke University and since 1970, at the University of Alabama in Birmingham. Using that ingenious preparation of isolated, perfused tubular segments, Dr. Schafer and his associates have made important contributions to our understanding of the epithelial transport of solutes and water. Like Dr. Pitts, Dr. Schafer is a gifted teacher who has four times been selected for Outstanding Teacher Awards at the University of Alabama in Birmingham. Also like Dr. Pitts, Dr. Schafer is recognized as an outstanding renal physiologist; he is editor of the* American Journal of Physiology: Renal, Fluid and Electrolyte Physiology. *He also serves on the editorial boards of several other journals. Dr. Schafer is currently Professor of Physiology and Biophysics and Senior Scientist in the Nephrology Research and Training Center at the University of Alabama in Birmingham.*

Heinz Valtin, Chairman, Renal Commission

In their classical experiments, Walker et al [1] and Gottschalk and Mylle [2] observed that fluid collected from the proximal tubule of various mammals remained apparently isosmotic to systemic plasma, despite rapid volume absorption. Similar observations were made in other epithelia such as the gall bladder [3] in which large transepithelial flows occurred in the absence of measurable differences in either osmotic or hydrostatic pressure across the tissue. By direct collection of absorbate from the gall bladder, Diamond [3] showed that the osmolality of this fluid was always within 5% of the osmolality of the luminal solution even when the osmolality of the latter solution was varied over a wide range.

To explain isosmotic volume absorption without invoking active water transport, a thermodynamic impossibility, the idea of an intraepithelial region of hyperosmolality was developed. This idea had its origins in the theoretical and experimental formulation of the middle or third compartment model [4–6]. This model demonstrated that fluid could move between two external fluid compartments in the absence of or even against an osmotic gradient if there were a middle compartment that was hyperosmotic to the external compartments, and if the opposing barriers of that compartment had different water-to-solute selectivity properties. It was suggested by Whitlock and Wheeler [7] that the lateral intercellular spaces within an epithelium could be the morphological counterpart of the hypothetical middle compartment. This concept was formalized by Diamond and Bossert [8] in their "standing gradient" hypothesis. In simplest terms, this hypothesis proposed that lateral intercellular spaces have a gradient of osmolality along their length, from hyperosmolar at the apical end to isosmotic at the basilar end. The development of the standing gradient, however, depends on the existence of sufficient diffusion constraints

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[1]This lecture was presented at the XXIXth Congress of The International Union of Physiological Sciences, Sydney, Australia, August 29, 1983.

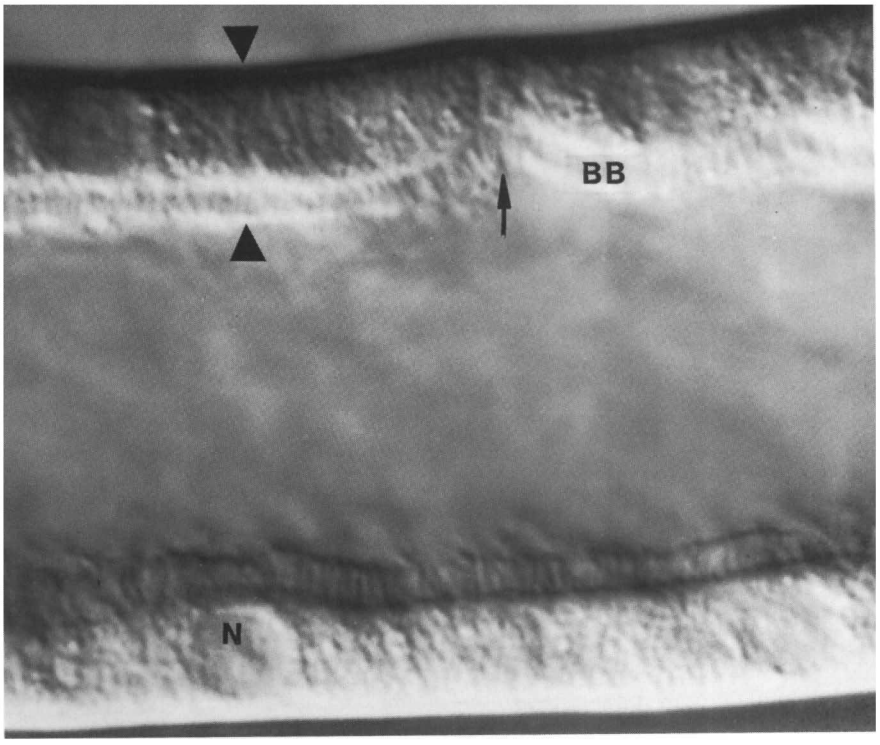


Fig. 1. *Differential interference contrast image of a rabbit proximal straight tubule from superficial cortex.* The tubule was bathed in a solution similar to serum and perfused with an ultrafiltrate of the same. Structures identified are: a nucleus (N) with a nucleolus apparent and the brush border (BB). The *dark arrowheads* denote the thickness of the total transport pathway which is 6.8 μm at the *point* indicated. The *lighter arrow* denotes the junction between two adjacent cells (Kirk and Barfuss, unpublished observations).

within the epithelium such that the hyperosmolality of the space is not dissipated by solute diffusion out of the basilar ends. Thus, the standing osmotic gradient is a steady-state balance of active solute pumping into the intercellular space, osmotic water flow into the space, and convective and diffusional movement out of the space.

Lateral intercellular spaces in the proximal tubule

The application of the standing gradient hypothesis to the proximal tubule has been questioned primarily because of the apparent lack of sufficient diffusion resistance within lateral intercellular spaces. Figure 1 is a micrograph of a rabbit proximal straight tubule during in vitro perfusion. The micrograph was made using Nomarski (differential interference contrast) microscopy [9] while the tubule was actively absorbing fluid. Using this methodology considerable structural detail can be observed including nuclei, nucleoli, the brush border, and intracellular vacuoles. What I want to point out by means of this micrograph is that diffusion distances, either in isolated tubules or in vivo, are extremely short. The total thickness of the epithelium, indicated by the dark arrowheads in Figure 1, is only 6 to 7 μm, and the luminal diameter is on the order of 20 μm. In contrast to many other epithelia, there is no supporting serosal tissue, and, in vivo, the peritubular capillaries are immediately juxtaposed to the basement membrane. Therefore, the total distance from the site of absorption of solutes at the luminal membrane to the capillary, is extremely short. This anatomic arrangement precludes the development of any significant gradients of solute concentration, either within the lumen itself or in the peritubular medium immediately adjacent to the epithelium. In Figure 1, the suggestion of intercellular spaces between adjacent cells can be seen at points as indicated by the arrow. The question then is whether the spaces between adjacent cells of the proximal tubule could provide a sufficient diffusion resistance to develop a standing osmotic gradient.

The lateral intercellular spaces can only be resolved using electron microscopy, by which they appear to be quite narrow (on the order of 300 Å in width) and apparently very tortuous. Both the restricted width and tortuosity could impede diffusion of solutes out of the spaces. However, as shown by morphometric analyses of transmission electron micrographs by Welling and Welling [10] and Welling, Welling, and Hill [11], the lateral membrane of the mammalian proximal tubule cell is highly folded and interdigitated with the lateral membranes of neighboring cells in three-dimensional space, giving the appearance of tortuosity in a two-dimensional transmission electron micrograph. These results have been confirmed qualitatively by the scanning electron micrographs of Evan et al [12]. Because of the highly infolded conformation of the lateral membrane, the cell circumference increases from 60 to 90 μm near the junctional complex to approximately 1400 μm at the basilar end. In addition, the three-dimensional conformation of the spaces is such that from any point at the junctional complex there is a straight-line path, not more than 7 μm in length, through the lateral intercellular space to the basement membrane. Thus, morphometric studies show that the lateral intercellular spaces of the proximal tubule are not tortuous and occupy a relatively large fraction of the cross-sectional area of the epithelium, especially near the base of the cell. Consequently, it can be demonstrated that no significant solute concentration gradient would exist along the spaces.

The low transepithelial resistance of the proximal tubule is also an indication of the relative lack of diffusion constraints within the lateral intercellular space. It has been shown that at least 90% of the high transepithelial electrical conductance in the mammalian proximal tubule is attributable to ionic move-

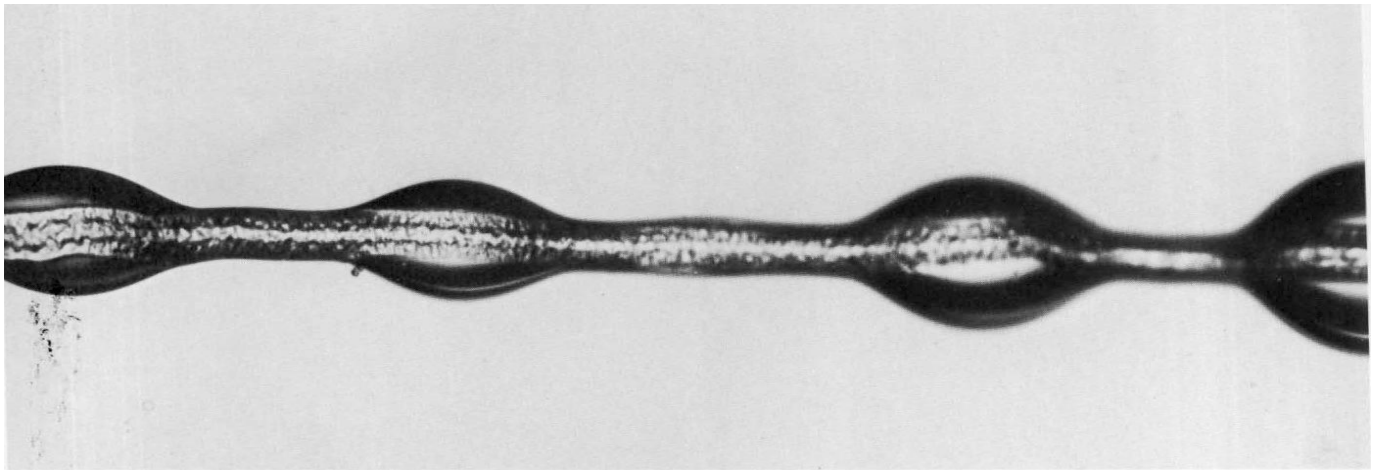


Fig. 2. *Micrograph of an isolated rabbit proximal tubule perfused under light mineral oil at 38°C.* Four absorbate droplets are seen on the peritubular surface where they are intermittently removed for analysis. The outside diameter of this tubule segment is 35 μm.

ment through the paracellular pathway, that is, the junctional complexes serially aligned with the lateral intercellular spaces [13]. Even if one attributes the entire transepithelial resistance to diffusion constraints within the space, we calculate that these diffusion constraints would be insufficient to establish significant hypertonicity [14, 15][1].

The conclusion reached from both theoretical and structural analyses of this type is that the lateral intercellular spaces can offer little diffusion resistance, and consequently could not accommodate the postulated standing gradient. Since the required osmotic driving force for volume absorption cannot be within the epithelium, one is led to conclude that these osmotic driving forces must exist between the external solutions. This would appear to contradict the whole concept of isosmotic volume absorption. However, experiments both in the rat in vivo and in isolated perfused rabbit proximal tubules have indicated that the transepithelial hydraulic conductivity is extremely high. This means that the required osmotic driving forces for volume absorption are relatively small.

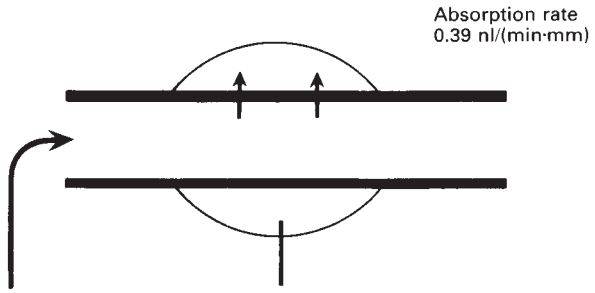
Hydraulic conductivity of the proximal nephron

The hydraulic conductivity or osmotic water permeability has been measured in proximal tubules by the rate of transepithelial volume flow observed in the presence of an osmotic gradient, as reviewed recently by Berry [16]. Both in the rat proximal convoluted tubule in vivo (for example, [17–20]) and in the isolated rabbit proximal convoluted tubule [21–23], most investigators have determined the osmotic water permeability to be in the range of 2,000 to 3,500 μm/sec. When this osmotic water permeability is converted to the units of hydraulic conductivity, it indicates that a volume absorption rate of 0.2 to 0.3 nl min^{-1} mm^{-1} could be driven for each milliosmole of osmotic pressure difference across the epithelium. On the other hand, hydrostatic pressure differences would not be expected to play a very large role in the production of volume absorption, since only approximately 0.01 nl/min flow would occur per millimeter of mercury of pressure difference. In other words, it is possible that very small transepithelial osmolality differences may be responsible for driving volume absorption in the proximal nephron. The problem has been to demonstrate these small osmolality differences with existing techniques for measuring the osmolality of nanoliter samples. Consequently, the hypothesis has remained untested. In the remainder of this paper, I will discuss new evidence showing that small osmolality gradients do develop across the proximal tubular epithelium. In addition, the contributions of solute reflection coefficient differences and sodium chloride diffusion to volume absorption in the late proximal tubule will be considered.

Direct collection and analysis of proximal tubule absorbate

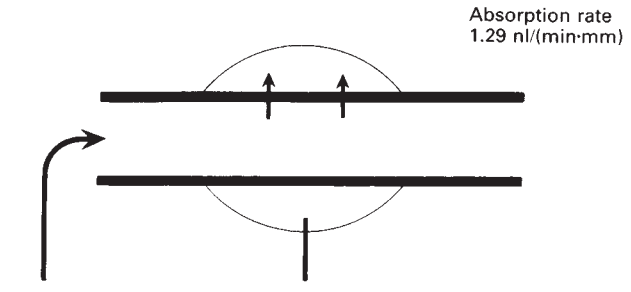
The difficulties inherent in demonstrating small transepithelial osmolality differences have been somewhat lessened by using a new technique developed by Delon Barfuss [24]. An isolated segment of rabbit proximal convoluted or proximal straight tubule is mounted between sets of glass micropipets and perfused in the standard manner. After perfusion has been initiated, the aqueous bathing solution is replaced by a layer of light mineral oil so that the tubule and the pipets are completely immersed in the oil. It is then observed that as the tubule is warmed to 38°C, absorbate begins to form on the peritubular surface as the small droplets shown in Figure 2. These droplets can be quantitatively collected for analysis using a constant volume pipet. The primary advantage of this technique is that the absorbate composition or osmolality can be compared directly to that of the original perfusate or of the collected perfusate that has passed through the tubule lumen (referred to in this paper as *collectate*). In these experiments, the perfusion rate is kept relatively rapid so that there is little change in the

[1] Although the evidence for low diffusion constraints is presently quite compelling, two reservations must be considered. First, due to the close apposition of two membranes bounding the lateral intercellular space, and the fact that both membranes are likely to have surface charge, surface electrical conductance may give a lower apparent resistance than that expected from the aqueous solution diffusion resistance. Second, although there is insufficient area restriction and no tortuosity of the intercellular spaces, if the solute diffusion coefficient within the spaces is significantly lower than in free solution (perhaps due to the effects of surface proteins on the lateral membrane surfaces), the diffusion constraints imposed by the spaces could be larger than expected.



Perfusate		Absorbate	
294.4	mOsm	302.1	($P < 0.02$)
148.0	Na^+	152.4	($P < 0.001$)
1.1	Glucose	4.5	($P < 0.001$)

Fig. 3. *Hyperosmolality of absorbate from rabbit proximal straight tubules.* A segment of the perfused tubule under oil is shown schematically with a droplet of absorbate on the peritubular surface. The composition of the perfusate, shown on the left, is the average of measured perfusate and collectate concentrations. Concentrations indicated for Na^+ and glucose are in millimolar. *P* values indicate the significance of the paired difference in average perfusate and absorbate compositions. Data are from Barfuss and Schafer [24–26].



Perfusate		Absorbate	
291.1	mOsm	306.9	($P < 0.001$)
147.2	Na^+	142.6	($P < 0.02$)
4.4	Glucose	29.9	($P < 0.001$)

Fig. 4. *Hyperosmolality of absorbate from rabbit proximal convoluted tubule.* Absorption under oil is shown schematically as described in the legend to Figure 3, and data are from Barfuss and Schafer [26, 27].

composition of the perfusate along the length of the tubule. In addition, a microelectrode can be introduced into an absorbate droplet so that the transepithelial voltage can be measured between this electrode and the inner perfusion pipet.

In addition to measuring the osmolalities of the absorbed and luminal fluid, which were the most important determinations in these studies, we also measured the following parameters. First, the rate of volume absorption was measured both by the rate of appearance of absorbate on the peritubular surface and the difference between the rates of perfusion and collection. Second, the transepithelial voltage was measured. Third, sodium concentrations in the absorbate and collectate, as well as the sodium transport rate were measured by adding $^{22}Na^+$ to the perfusate. Finally, glucose concentrations and transport rate were measured in the same way by the addition of ^{14}C glucose to the perfusate.

Figure 3 presents a composite of results obtained by analysis of absorbate from proximal straight tubules [24–26]. These tubules were perfused with a standard solution resembling an ultrafiltrate of serum and containing bicarbonate, glucose, and amino acids. We refer to this as a complete perfusate. With this perfusate, the volume absorption rate at 38°C was 0.35 to 0.40 nl min^{-1} mm^{-1}. We then measured the osmolality and the concentrations of sodium and glucose, both in the perfusate and the absorbate collected from the peritubular surface. There was a significant difference between the osmolality of the perfusate and that of the absorbate; the absorbate was hyperosmotic to the perfusate by approximately 8 mOsm/kg H_2O. The higher osmolality of the absorbate could be accounted for, first, by a higher concentration of sodium and its accompanying anions, and second, by a higher concentration of glucose. Therefore, these results indicate that the proximal straight tubule is capable of developing a hyperosmotic absorbate due to active transport of both sodium and preferentially absorbed solutes such as glucose.

As shown schematically in Figure 4, the same experiments were also performed in the rabbit proximal convoluted tubule perfused with the complete perfusate under a layer of light mineral oil [26, 27]. In these segments, the rate of volume absorption at 1.2 to 1.4 nl min^{-1} mm^{-1} was higher than observed in proximal straight segments and the same as during perfusion with a normal aqueous bathing solution containing protein [28, 29]. It can be seen from the results in Figure 4 that the absorbate was significantly hyperosmotic to the luminal perfusate by over 15 mOsm/kg H_2O. However, in marked contrast to the proximal straight tubule, the sodium concentration in the absorbate from the proximal convoluted tubule was actually lower than the sodium concentration in the lumen. Thus, other solutes must contribute significantly to the hyperosmolality of the absorbate from the convoluted segment. A prime contributor is glucose, because a transepithelial concentration gradient in excess of 25 mM was observed. Therefore, in the proximal convoluted tubule, a large fraction of the absorbate osmolality is due to glucose and probably other preferentially absorbed solutes.

To illustrate this point, we also performed experiments in which the glucose concentration in the perfusate was reduced from 7.4 to 5 or 1 mM, and the amino acid concentration was decreased to 0.5 mM. We observed a direct reduction of volume absorption with a decreasing concentration of preferentially absorbed solutes in the perfusate as observed previously by Burg et al [28]. However, there was a direct correlation between the rate of volume absorption and the measured difference in osmolality between the luminal fluid and absorbate. From the slope of the relation between volume absorption rate and transepithelial osmolality difference in the convoluted tubule, we calculated that the osmotic water permeability is approximately 800 μm/sec. Although this value is close to some estimates obtained recently in the rat proximal convoluted tubule [16], it is only 25 to 35% of the value that we measured in isolated perfused rabbit proximal convoluted tubules bathed in aqueous solution [22]. From the volume absorption rate and osmolality gradient observed in the straight segment (Fig. 3), we computed that the osmotic water permeability of this segment is also considerably lower than previously measured in aqueous bathing solutions [22]. We presently have no explanation for the apparent lower hydraulic conductivity, unless it relates to the effect of the oil on the configuration of the lateral intercellular space, or to the fact that these tubules were examined at 38°C rather than at 23°C, as in the previous study [22].

Luminal hypotonicity

The experiments with both proximal convoluted and straight tubules perfused under oil demonstrate that a hyperosmotic absorbate can be formed as a consequence of active solute absorption. However, the situation with the tubule perfused under oil is different than the in vivo situation in that the volume of the peritubular compartment is limited to the absorbate itself. Consequently, active solute absorption can generate a hyperosmotic absorbate. How does this apply to the normal in vivo situation, or the situation with an isolated tubule perfused in an aqueous bathing solution? In both cases we have argued that the peritubular compartment must remain isosmotic, but we believe that active solute absorption operates to develop a transepithelial osmolality difference.

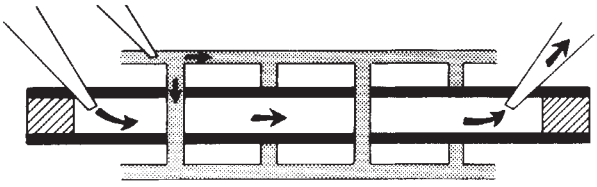
Our hypothesis is that solute absorption tends to produce luminal hypotonicity [30, 31]. In vivo, or in the case of isolated tubules perfused in an aqueous solution, the peritubular fluid remains isosmotic due to rapid capillary perfusion. Therefore, rather than raising peritubular osmolality, solute absorption should tend to lower the luminal osmolality until the resulting osmolality difference produces a water flow sufficient to match the solute flow, and in the steady-state the absorbed fluid should actually be slightly hypoosmotic to plasma.

This hypothesis has been tested recently by the experiments of Green and Giebisch [32]. In these experiments, illustrated in Figure 5, a segment of rat proximal convoluted tubule was perfused in vivo between oil blocks at either end of the segment. At the same time, peritubular capillaries were perfused at a rapid rate, so as to maintain a constant and known composition in the peritubular space. The perfusate for both the lumen and the peritubular capillaries in these experiments was a simple sodium chloride solution with an osmolality of 290 mOsm/kg H_2O. Green and Giebisch then measured the rate of volume absorption and the osmolality difference between the collected fluid and the perfused fluid at two different perfusion rates and in the presence of cyanide as an inhibitor of active solute absorption. Although the rates of volume absorption they observed were lower than in the presence of a more physiologic perfusate, they did demonstrate a significant transepithelial osmolality difference. The osmolality difference indicated in Figure 5 showed that the luminal perfusate became dilute. Furthermore, when the volume absorption rate was quickened by increasing the perfusion rate, the osmolality difference rose in proportion. However, when cyanide was added, both the volume absorption rate and the osmolality difference were reduced to zero.

In summary, the experiments of Green and Giebisch [32] demonstrate that a transepithelial osmolality gradient can be developed by dilution of the luminal perfusate due to solute absorption. However, in the case of the isolated tubule perfused under oil, the transepithelial osmolality gradient can be developed by a hyperosmotic absorbate. In both cases, the development of this gradient is produced by active sodium absorption, but it is greatly augmented by preferential absorption of solutes such as glucose.

Effects of preferential solute absorption

In the experiments shown in Figures 3 and 4, the *absolute* osmolality difference between luminal perfusate and peritubular solution was measured. By absolute osmolality, I refer to the



Perfusate: 154 mM NaCl (290 mOsm)

Perfusion rate	Reabsorption rate	Osmolality difference (Collectate-Perfusate)
nl/min	*nl/(min·mm)*	*mOsm*
10	0.41	− 1.73 ± 0.48
45	0.89	− 3.90 ± 0.64
45 + NaCN	− 0.06	0.47 ± 0.38

Fig. 5. *Development of luminal hypotonicity in the perfused rat proximal nephron in vivo.* Simultaneous perfusions of the rubule lumen and peritubular capillary network are shown schematically. The results are for differing tubule perfusion rates in the absence or presence of NaCl. Reabsorption rates of tubular perfusate and the differences in osmolality between collected and perfused tubular fluid are given. Data are from the work of Green and Giebisch [32].

osmolality that would be measured by a method such as freezing point depression. However, this measurement does not consider the effects of solute reflection coefficient differences in producing a transepithelial osmolality difference. These factors are particularly important when one considers that normally the composition of the tubular fluid varies quite markedly from that of the peritubular solution along the length of the proximal tubule. Due to preferential, active absorption, the tubular fluid concentrations of bicarbonate, glucose, and amino and organic acids decrease along the length of the proximal nephron [31, 33, 34]. On the other hand, the concentration of chloride rises in proportion to the fall in bicarbonate concentration. Thus, tubular fluid toward the latter regions of the proximal tubule has a composition quite different from that of the plasma or interstitial fluid. These transepithelial solute concentration differences might serve to produce what has been referred to as effective osmolality differences [30].

For each solute the effective osmolality difference is equal to the product of its reflection coefficient (σ) and the difference in concentration across the epithelium (ΔC). When we consider the several solutes that comprise tubular and interstitial fluids, the total effective osmolality difference is given as the sum of these products:

$$\text{Total effective osmolality difference} = RT\Sigma\sigma_i \cdot \Delta C_i \quad (1)$$

The importance of an effective osmolality difference in producing volume absorption is that regardless of the measured absolute luminal osmolality, effective luminal hypoosmolality relative to the peritubular interstitial fluid can produce volume

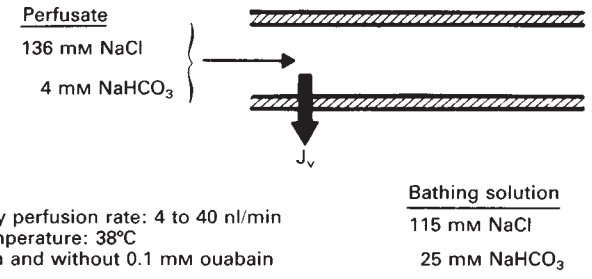


Fig. 6. *Design of experiments to demonstrate the effect of perfusion rate on volume absorption produced in the presence of opposing Cl^- and HCO_3^- gradients across the epithelium.* An isolated proximal straight tubule is shown schematically, and the experimental conditions are indicated. See [35] for details.

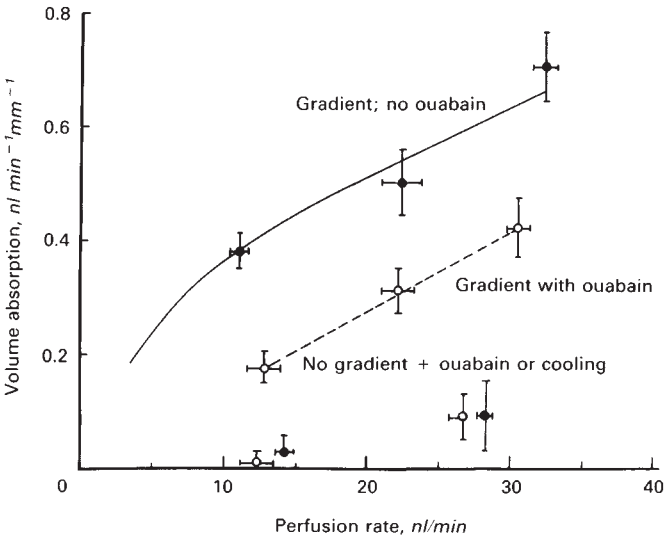


Fig. 7. *Flow dependence of volume absorption in rabbit proximal straight tubule.* For the upper two sets of data, tubules were perfused with the high Cl^- perfusate while bathed with the normal Cl^- concentration as indicated in Figure 6. The lowest data points were obtained when Cl^- and HCO_3^- concentrations were equal in the perfusate and bathing solution. Data are from Schafer et al [35].

absorption. The preferential absorption of bicarbonate and organic solutes with high reflection coefficients can produce the effective luminal hypotonicity. As an example, consider the effect of transepithelial HCO_3^- and Cl^- gradients in the proximal tubule. Due to preferential bicarbonate absorption, the bicarbonate concentration in the lumen of the late proximal tubule is about 20 mM lower than in the peritubular medium. To maintain electroneutrality, the Cl^- concentration is elevated by the same amount. If we presume that the reflection coefficients of bicarbonate and chloride differ by only 0.2, then, according to equation 1, there would be a significant effective osmolality gradient with the lumen dilute by 4 mOsm/kg H_2O relative to the bathing solution. Given the high osmotic water permeability of the proximal tubule, this difference would be sufficient to drive volume absorption at the respectable rate of 1.0 nl min^{-1} mm^{-1}.

In the same way, the preferential absorption of organic solutes such as glucose and amino acids could also lead to the development of an effective osmolality difference. The total organic solute composition of an ultrafiltrate of rat or rabbit plasma is approximately 12 mM. At the end of the proximal tubule, after these organic solutes have been almost completely absorbed, the sum of their concentrations is consequently 12 mM less than in the peritubular solution. To balance the fall in organic solute concentration, the concentration of sodium and chloride must rise to an equal extent. If we hypothesize that the reflection coefficients of organic solutes such as glucose and amino acids are approximately 1, but that of sodium chloride is 0.8, then the effective osmolality difference due to the organic solutes exceeds that of sodium chloride, giving a net transepithelial osmolality difference of about 2.4 mOsm/kg H_2O. Again, given the high hydraulic conductivity of the proximal nephron, even this small effective osmolality difference could produce a volume flow of about 0.6 nl min^{-1} mm^{-1}.

As shown in Figure 6, we designed experiments to demonstrate the existence of an effective osmolality difference produced by bicarbonate and chloride gradients [14, 35]. In these experiments, the bathing solution contained a normal sodium bicarbonate concentration, but in the perfusate bicarbonate was almost completely replaced by chloride. Thus, there were opposing chloride and bicarbonate gradients such as those that exist in the late proximal tubule. The two solutions had an identical absolute osmolality of 290 mOsm/kg H_2O. These proximal straight tubules were then perfused at rates of 4 to 40 nl/min in the presence and absence of ouabain. In Figure 7, the rate of volume absorption in these experiments is plotted as a function of the perfusion rate. The uppermost data points show the volume absorption observed when the chloride and bicarbonate gradients were present without ouabain. There was substantial volume absorption which increased as the perfusion rate increased. When active volume absorption was inhibited by the addition of ouabain, as shown by the data points in the middle curve, the rate of volume absorption fell, but it was still significantly greater than zero. However, when the anion concentration gradients were removed, as shown at the bottom of the graph, so that there were equal concentrations of chloride and bicarbonate in both the perfusate and bathing solution, and active transport was inhibited by ouabain or cooling, there was no volume absorption at either a low or a high perfusion rate. In the following discussion, I will concentrate on the middle data points that show volume absorption in the presence of anion gradients, but in the absence of active transport. This process has been referred to as "passive volume absorption" [14, 35].

Rector et al [36] proposed two mechanisms by which volume absorption, in the presence of the anion gradients, might continue when active transport is inhibited. First, volume absorption could continue as a consequence of sodium and chloride diffusion down their respective electrochemical potential gradients, if this rate of diffusion were more rapid than the rate of sodium bicarbonate diffusion back into the lumen. The loss of solute from the lumen would be followed by an equivalent loss of water. Second, the luminal perfusate can be effectively hypoosmotic due to the fact that the chloride reflection coefficient is less than that of bicarbonate. Both mechanisms have been proposed to contribute about equally to sodium chloride absorption according to the results of Frömter, Rumrich, and Ullrich [37] and Neumann and Rector [34]. Warner and Lechene [38, 39] have argued that both mecha-

nisms, that is, passive sodium chloride diffusion and effective luminal hypotonicity, are far more important than active Na^+ absorption in producing net volume absorption from the split droplet in the rat proximal tubule.

To test the hypothesis for a difference in the bicarbonate and chloride reflection coefficients, Jacobson et al [40] and Corman and DiStefano [41] attempted to demonstrate solvent drag of sodium and chloride by imposing an osmotic gradient in the absence of active transport. In these experiments, they were unable to demonstrate any significant enhancement of net solute transport that could be attributed to solvent drag. These results imply that water and solute traverse different pathways across the epithelium, and therefore one would not expect that sodium chloride would have a reflection coefficient of less than one. Certainly these results preclude the existence of very low reflection coefficients, but are the methods accurate enough to determine reflection coefficients in the range of 0.8? In the example discussed above, if the HCO_3^- reflection coefficient were approximately 1.0 and that of Cl^- were 0.8, a significant osmotic driving force could be developed. But what would be the effect of the water flow on chloride movement? The solvent drag component of the Cl^- flux would be given as:

$$\text{Chloride solvent drag} = J_v (1 - \sigma_{Cl}) \bar{C}_{Cl} \qquad (2)$$

where: J_v is the volume absorption rate (nl min^{-1} mm^{-1}), σ_{Cl} is the chloride reflection coefficient, and $\bar{C}_{Cl}$ is the mean chloride concentration in the solutions. In the experiments of Jacobson et al [40] and Corman and DiStefano [41], the maximum passive J_v generated was less than 1.8 nl min^{-1} mm^{-1}, but I will use this value as an upper bound to *overestimate* the possible Cl^- solvent drag. The average chloride concentration was about 110 mM (110 pmoles/nl). Thus, for $\sigma_{Cl} = 0.8$, the solvent drag of Cl^- would amount to less than 40 pmoles min^{-1} mm^{-1}. This solvent drag flux must be contrasted with the rate of Cl^- perfusion, which was lowest in the experiments of Corman and DiStefano [41] who perfused tubules at 9 nl/min with a chloride concentration of 120 mM, giving a Cl^- delivery rate of 1,080 pmoles/min. Thus, for the typical proximal convoluted tubule length of 1 mm, it would have been extremely difficult to observe a change in Cl^- flow in the lumen of 40 pmoles/min or less than 4% of the perfusion rate, given the usual accuracy of determining both volume flow rates and chloride concentrations or osmolality. Thus, the conclusions of Jacobson et al [40] and Corman and DiStefano [41] are correct in that solvent drag probably comprises at best a small fraction of total solute absorption in the proximal tubule. However, as noted above, a Cl^- reflection coefficient of 0.8, which would be difficult to demonstrate by these techniques, could generate a significant effective osmotic pressure difference.

Since this analysis indicates that reflection coefficients on the order of 0.8 are consistent with the absence of significant solvent drag, it appears that the maintenance of volume absorption observed in the presence of ouabain (Fig. 7, middle data points) could be due either to reflection coefficient differences or to sodium chloride diffusion. I will first consider the possibility that passive sodium chloride diffusion out of the lumen is solely responsible for the volume flow, and that the reflection coefficients for Na^+, Cl^-, and HCO_3^- are all unity. Since the reflection coefficients are all taken to be 1.0, there is no contribution of solvent drag to either Na^+ or Cl^- diffusion, and the net flux of each ion can be approximated as [35, 37]:

$$J_{Na} = P_{Na} \cdot \left[\Delta C_{Na} + \frac{F}{RT} \bar{C}_{Na} V_e \right] \qquad (3)$$

$$J_{Cl} = P_{Cl} \cdot \left[\Delta C_{Cl} - \frac{F}{RT} \bar{C}_{Cl} V_e \right] \qquad (4)$$

where P is the permeability of Na^+ or Cl^-, ΔC is the transepithelial concentration difference (lumen-bath), $\bar{C}$ is the average of the luminal and peritubular ion concentrations, V_e is the transepithelial voltage (lumen with respect to bath), F is the Faraday constant, R is the gas constant, and T is the absolute temperature.

Table 1 [42] gives the values used in the analysis of the Na^+ and Cl^- fluxes. In the case of sodium, in accordance with micropuncture observations, it is assumed that there is no transepithelial concentration gradient and that the flux of sodium would be driven primarily by lumen positive voltage. On the other hand, the luminal Cl^- concentration is on the order of 20 mM higher than in the peritubular medium, and its absorption would be driven primarily by the concentration gradient and opposed by the voltage. Since we are interested in the maximum rate of volume absorption that could be produced by sodium chloride diffusion out of the tubule in the experiment shown in Figures 6 and 7, it is assumed that the fluxes of Na^+ and Cl^- must be equal. In other words, we combine equations 3 and 4 by the condition of electroneutrality that $J_{Na} = J_{Cl}$. It is then possible to solve the combined equation for the unique transepithelial voltage V_e that will give $J_{Na} = J_{Cl}$. This voltage is calculated to be +3.4 mV, which is approximately the voltage observed in these experiments [13, 34]. Using this value in equation 3 or 4, the predicted Cl^- and Na^+ fluxes are 19.3 pmoles min^{-1} mm^{-1}. To compute the volume flow, J_v, that would accompany the salt movement, the sum of the net fluxes is divided by the isosmotic concentration of 290 mOsm/kg H_2O to arrive at a predicted volume flow of 0.13 nl min^{-1} mm^{-1}. This rate of volume flow is far less than the volume flows observed in the presence of anion concentration gradients in the experiments shown in Figure 7, especially at the higher perfusion rates, and demonstrates that simple sodium chloride diffusion from the lumen would be insufficient to explain more than a fraction of the volume absorption. Nevertheless, there is no question that sodium chloride diffusion out of the lumen would contribute a small amount to volume absorption in the late proximal tubule.

The alternative hypothesis is that the chloride reflection coefficient is less than that for bicarbonate. If this is true, then in the experiments in which opposing Cl^- and HCO_3^- gradients are imposed, the luminal perfusate is effectively hypoosmotic to the bathing solution. This is illustrated in Figure 8 by a theoretical analysis of the flow dependence of volume absorption in the experiments in which tubules were perfused with the high chloride solution and bathed in the bicarbonate-containing solution in the presence of ouabain. These theoretical results were obtained by assuming that the $NaHCO_3$ reflection coefficient was 1.0, and that the sodium chloride reflection coefficient was 0.8 [35]. In Figure 8, the predicted volume absorption rate and the absolute luminal osmolality are plotted as a function of

Table 1. Parameter values used to calculate Na^+ and Cl^- fluxes

Parameter	Na^+	Cl^-
P, $\mu m/sec$[a]	0.23	0.73
pmoles min^{-1} mm^{-1} mM^{-1}	1.04	3.28
ΔC, *mM*	0	20
$\bar{C}$, *mM*	145	110

[a] Permeabilities are those measured in superficial rabbit proximal straight tubules by isotopic flux and electrophysiologic methodology [42]. The conversion of permeability units was made assuming an inside tubule diameter of 23 μm.

length along the perfused tubule segment. Although the luminal osmolality at the perfusion end of the tubule is 290 mOsm/kg H_2O, which is the same as that of the bathing solution, it is effectively hypoosmotic relative to the bathing solution because of the lower reflection coefficient of chloride. In this situation, osmotic equilibration due to net water efflux will be achieved after a length of about 0.5 mm. However, when the perfusion rate is elevated to 30 nl/min, osmotic equilibration is delayed along the tubule length and is achieved only after about 2 mm. Consequently, the rapid volume absorption occurs along a longer length of the perfused segment. In the first column in the upper right area of Figure 8, the average volume absorption in this 2.5 mm length of perfused tubule is taken from the integrated area under the volume absorption curve divided by the tubule length. The second column gives the volume absorption rate that was actually measured in these experiments. It can be seen that quite good agreement exists at both the low and the high perfusion rates.

Both the experiments in Figure 7 and the theoretical evaluation of these experiments in Figure 8 demonstrate quantitatively that reflection coefficient differences, although quite small, do play a significant role in the development of an effective osmotic gradient across the proximal tubular epithelium. Nevertheless, the resulting effective osmolality difference cannot drive truly passive volume absorption because it is dissipated by water flow, which tends to produce osmotic equilibrium rapidly as noted in Figure 8. Only a small residual volume absorption rate that is observed after osmotic equilibration has occurred can be considered to be true passive volume absorption. This volume absorption is, in fact, that small amount which may be attributed to the diffusive movement of sodium chloride out of the lumen, as calculated from equations 3 and 4 above. However, in the situation in which active transport is continuing, equilibration of the effective luminal osmolality with the bathing solution does not occur because of continued active solute removal from the lumen. Our conclusion is that active transport is still the dominant force in driving volume absorption in the late proximal tubule, but that reflection coefficient differences may produce absolute hyperosmolality but effective hypoosmolality of the luminal fluid in the late proximal nephron. The most important test of this hypothesis will depend on the demonstration of the predicted rise in the absolute luminal osmolality as a consequence of volume movement in experiments such as these. This remains an important task for the future.

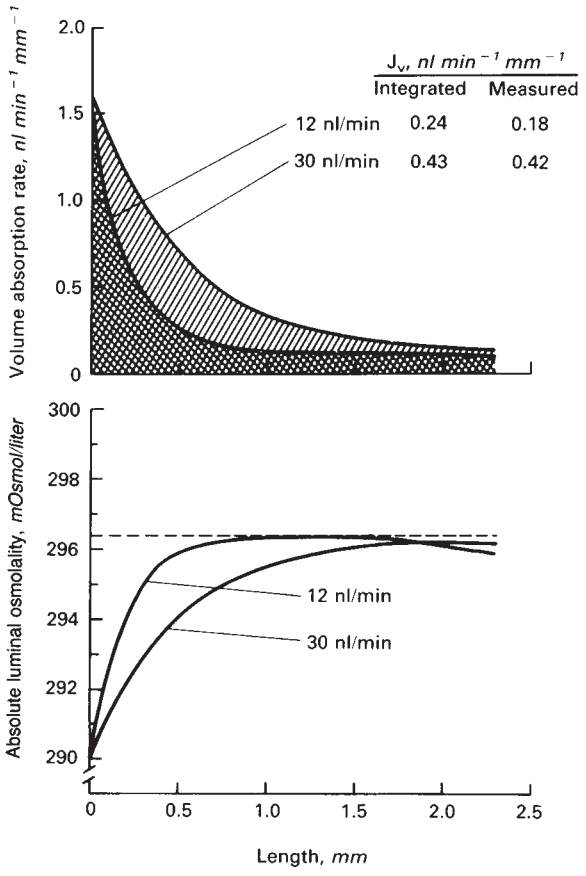


Fig. 8. *Theoretical predictions for the dependence of volume absorption on the perfusion rate.* Conditions for the model were the same as the experimental conditions of Figure 6 with ouabain present. Volume absorption rate is computed as the area under the theoretical curves and is compared with that measured in the experiments shown in Figure 7 *(middle curve)*. See text and [35] for details. (Reprinted with permission from *Kidney International*)

Summary

The recent studies cited above show that a transepithelial osmolality difference may be developed by solute absorption in the proximal nephron. Although small, this osmolality difference can drive normal rates of volume absorption due to the high hydraulic conductivity of the epithelium. Experiments in the rat proximal tubule in vivo have demonstrated dilution of the luminal perfusate by solute absorption. In isolated tubules perfused under oil, the absorbate has been directly examined and found to be hyperosmotic compared to the perfusate. In the early proximal tubule, the rapid active transport of sodium salts, as well as the preferentially absorbed solutes, such as bicarbonate, glucose and amino acids, produces absolute luminal hypoosmolality of the tubular fluid. In the late proximal tubule, where transepithelial gradients for bicarbonate and the organic solutes have been established by preferential absorption in the early segment, there may be absolute luminal hyperosmolality but effective hypoosmolality, because of the higher reflection coefficients for the preferentially absorbed solutes. However, the maintenance of effective hypoosmolality of the tubular fluid along the entire length of the proximal nephron still depends on continued active solute absorption. A small fraction of the volume absorption occurring in the proximal nephron can be attributed to sodium chloride diffusion out

of the lumen, and only this fraction can be regarded to be truly passive volume absorption.

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